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ROLE OF NANOPARTICLES IN THE EFFECTIVE REMOVAL OF BIOFILM: A NARRATIVE REVIEW

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ABSTRACT
The success of endodontic treatment is thorough debridement and cleaning of the root canal system. Endodontic treatment is considered challenging as the conventional cleaning and disinfection methods fail to eliminate persistent bacterial biofilms within the complex anatomical structures. Root canal infections are biofilm mediated. The complexity of the root canal system along with various bacteria in biofilm makes disinfection extremely challenging.
Traditional methods might fail to penetrate the complex root canal system leading to persistence of biofilm in lateral and accessory canals making it a niche for secondary infection. The advent of nanoparticles in the field of endodontics has shown noteworthy results. Nanoparticles possess antibacterial, antifungal and antiviral properties. They can cause the destruction of cell wall, increase the permeability of cell membrane, production of reactive oxygen species, inhibition of various enzymes and genotoxicity. Thus, nanoparticles could transform endodontics by obtaining greater results and improving the quality of success of treatment. Various nanoparticles like graphene, silver nanoparticles, chitosan, hydroxyapatite, bioactive glass, iron compounds, etc have been extensively studied. The future of endodontics is heading down the nano-direction as most of the challenges faced are all nanosized.

KEYWORDS: Bacteria; Nanoparticles; Biofilm; Irrigation; Root canal.

INTRODUCTION
A biofilm is a highly organized structure consisting of bacterial cells enclosed in a self-produced extracellular polymeric matrix attached on a surface.[1] They consist of microbes that are irreversibly attached to each other or surface, embedded in a highly organised matrix of extracellular polysaccharide.

Persistence of microbes is the most important cause for the failure of root canal treatment. The main goal of root canal treatment is to completely eradicate the microbes from the root canals. The complex and highly unpredictable nature of the root canal anatomy and multi-species of biofilm amplify the difficulty in the removal of microorganisms.[1]

Conventional irrigants such as NaOCl causes allergic reaction, tissue toxicity, staining of instruments, irritation to periapical tissue, inability to remove smear layer, and has an undesirable smell and taste. CHX when mixed with sodium hypochlorite produces a carcinogenic product, i.e., parachloroanaline. CHX has disadvantages of undesirable smell and taste as well as tissue toxicity.

To overcome the drawbacks of conventional methods in removal of microorganisms, nanoparticles with various advantages have come into action.

This review article focuses on the root canal biofilms and the role of nanoparticles in their removal.

STAGES IN THE FORMATION OF BIOFILM (Fig.1)

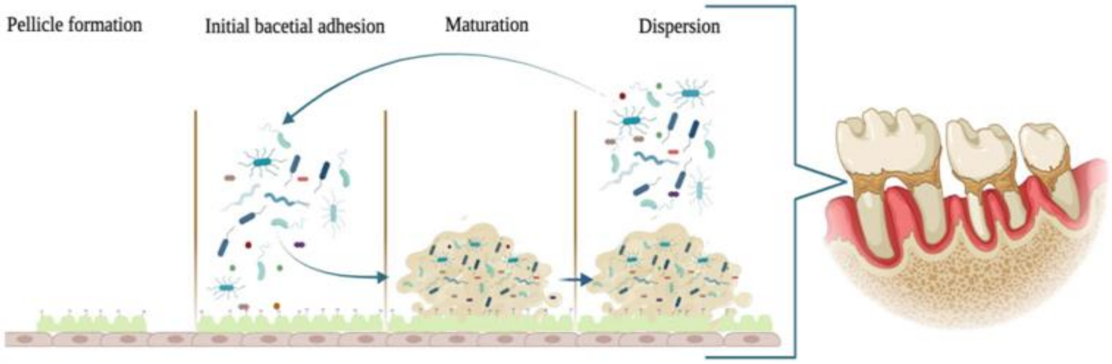


Figure 1. The oral biofilm formation stages: (1) salivary pellicle formation on the tooth surface; (2) adhesion of colonizing bacteria to the tooth through recognition of proteins present in the salivary pellicle; (3) biofilm formation and maturation; and (4) dispersion of planktonic bacteria from the matrix for new colonization.

Stage 1: Formation of pellicle on tooth surface
Stage 2: Attachment of bacteria on to the salivary pellicle
Stage 3: Maturation of biofilm
Stage 4: Dissemination of bacterial cells for further colonization

Characteristic features of a Biofilm

- **Protection of Biofilm Bacteria from Environmental Threats**

Extracellular secretions, such as extracellular polysaccharide or cell surface structures, like capsules, can be produced by the bacteria. For all resident bacteria, the extracellular polysaccharide (EPS) can provide protection against a range of external stress like osmotic shock, pH changes, and UV light. Thereby, the impact of any toxic compounds must diffuse through the EPS matrix before they can reach the bacteria.

- **Increased Tolerance to Antimicrobials**

Due to changed gene expression and the transfer of resistance genes, long-term antibiotic use causes bacteria to become resistant, making the antimicrobial agent ineffective.

- **Quorum Sensing**

Bacterial cells communicate with one another through a process called as quorum sensing. Bacteria can interact with each other through chemical signalling molecules. Quorum sensing enables bacteria to check their surroundings for other bacteria and adjust their behaviour on a population-wide scale.

- **Adherence to the surface**

Endodontic Biofilms

I. Intracanal biofilm

These are biofilms that are formed on the radicular dentin in an endodontically infected tooth[12]. *E. faecalis* is found to be the most prevalent bacteria in this type of endodontic biofilm.

II. Extra-canal biofilm

Microbial biofilms deposited on the root surface(cementum) adjacent to the root apex of endodontically infected teeth are known as extra-radicular microbial biofilms or root surface biofilms.[13]

III. Periapical biofilm

Isolated microbial biofilms located in the periapical area of endodontically infected teeth are known as periapical microbial biofilms.[13] *Actinomyces* and *P. propionicum* have shown to form periapical lesions resistant to endodontic therapy.[12]

IV. Biomaterial-centred biofilm

Foreign body-centred biofilm is found when bacteria adhere to an artificial biomaterial surface and form biofilm structures. It is also known as biomaterial-centred infection.[14]

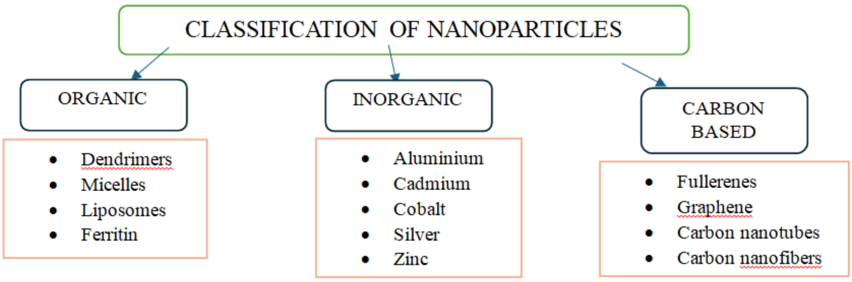
Significance of biofilm in Endodontics

The notion of endodontic microbiology is crucial for understanding the pathogenic potential of root canal microbiota and for laying the groundwork for novel infection control strategies since endodontic diseases are biofilm-mediated infection.

Understanding how root canal bacterial biofilms thwart endodontic treatment methods is crucial. In addition to providing bacteria with efficient defence against the host's system, the biofilm community increases the bacteria's resistance to a range of disinfectants. Both the removal of biofilms and the efficient killing of biofilm bacteria are essential for the successful treatment of these diseases.

As per the European Commission's Recommendation," Nanomaterial" is defined as a natural, incidental, or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions are in the range 1-100nm.[2]

NANOPARTICLES



Classification (Fig.2)

According to the Anil Kishen et al
Classification of nanoparticles Based on Composition
Inorganic -Zinc oxide, Iron oxide, Titanium dioxide, Cerium oxide
Metallic - Silver, Iron, Copper, Gold
Polymeric – Alginate, Chitosan
Quantum dots -Cadmium sulphide, Cadmium selenide
Functionalised-Drugs, Photosensitizers, Antibodies

Mechanism of action

➢ *Electrostatic interaction leading to cell membrane disruption*

Electrostatic contact causes cell membrane rupture. Positively charged nanoparticles react with the negatively charged surface of microorganisms leading to an increase in the permeability of cell causing cellular content leakage and also resulting in the buildup of NPs on the bacterial cell surface.[2] These positively charged NPs are efficiently linked to the cell membrane, upsetting the cell wall architecture and increasing the permeability of the cell, allowing more and more NPs to enter the bacteria, resulting in cellular content leakage. These nanoparticles, by attaching to mesosomes, impact respiration, division, and DNA replication.

➢ *Metal ion homeostasis*

Metabolic functions are dependent on homeostasis of metal ion present in the microbes. Excess of metal NPs causes irreversible damage leading to the retardation of growth or killing of microbes.

➢ *Production of reactive oxygen species*

NPs gain access to the microorganism's cell membrane and cause the release of ROS, resulting in oxidative stress in the cell and an attack on the bacterium. Due to this attack, respiration and ATP synthesis are reduced, causing rupture of the cell membrane. ROS is formed by a metal oxide through active redox cycling and the pro-oxidant functional group at the metal oxide-NP contact.

➢ *Dysfunction of protein and enzyme*

NPs cause the formation of carbonyls which results in the degradation of protein, inactivation of various enzymes and disruption of catalytic activity.[3]

➢ *Genotoxicity and inhibition of signal transduction*

NPs interact with nucleic acid molecules because of their electrical characteristics, which has a detrimental effect on chromosomal and plasmid DNA replication and inhibits signal transduction.

Properties of Nanoparticles

NPs are *less stable*, exhibit *weaker bonding* and interaction with other molecules. They also show *higher surface area to volume ratio* which helps them penetrate into tiny structures. NPs particles also cause disruption of cell wall synthesis and inhibit various enzymes.[2]

Various Nanoparticles used in endodontics to eliminate Biofilm

- ***Silver nanoparticles***

Silver is the most widely used metal nanoparticles for inhibiting different types of microorganisms and drug-resistant microorganisms. They can easily penetrate the bacterial cell because of their larger surface area and small size. Silver nanoparticles are found to be effective as irrigants, intracanal medicaments and root canal sealers due to their antibacterial activity . The antimicrobial activity is attributed to the release of Ag+ ions, increasing permeability and causing damage to the bacterial cytoplasm and DNA.

In contrast to the traditional method of irrigating root canals with sodium hypochlorite and EDTA sequentially, a single solution of NaOCl and AgNPs with chelating agents saves time and is less cytotoxic than widely used irrigants. They are also found to be more biocompatible than NaOCl at lower concentrations and are effective against E.faecalis.

- ***Graphene Nanoparticles***

Graphene NPs exhibit excellent stability, biocompatibility, electrical conductivity, biodegradability, mechanical properties and antimicrobial properties.

They prevent the colonisation of bacteria on root canal walls, leading to the success of endodontic treatments. Graphene oxide NPs are found to be effective in killing the growth of S.mutans. Graphene nanocomposites demonstrate excellent antibacterial activity by physically damaging the microorganisms by penetrating and breaking the cell membranes, along with wrapping the cells to induce mechanical stress and by extracting phospholipids from lipid membranes. It is also capable of producing oxidative stress.[5]

- ***Mesoporous calcium silicate***

These NPs are highly viscous in nature and has found to be effective in filling the apical third of the root canals. They also exhibit higher surface area to volume ratio, effective antibacterial property and are efficient in drug delivery.

MCSNs have also shown the ability to promote apatite mineralization, tissue regeneration and repair. CPC-loaded mesoporous silica nanocarriers have the advantage of high percentage loading, suggesting their potential application as drug delivery systems to combat biofilms and overcome numerous challenges such as delivery of drug molecules to intricate anatomical structures inside the tooth to provide the desired bactericidal properties.[10]

- ***Nanopolymers***

Quaternary polyethylene ammonium (QPEI) is a nanopolymer that exhibits insolubility, biocompatibility and chemical stability in contrast to other materials used in chemo mechanical root canal disinfection such as calcium hydroxide materials.[11] QPEI when added to the epoxy resin sealant, reduces the viability of E. faecalis in dentinal tubules, making endodontic treatment more effective and thereby reducing the complications caused by bacteria.

- ***Chitosan***

Chitosan exhibits excellent antibacterial, antifungal and antiviral properties. They cause disruption of bacterial cell membrane due to its electrostatic interaction. Chitosan can penetrate the root canal complexities and eliminate microorganisms. Chitosan particles attach to negatively charged cell wall of bacteria, release cellular contents and cause cell death.[6] It is also a chelating agent which chelates the metal ions present in bacteria and thereby interrupting many bacterial enzymes and their metabolism.[6] The use of chlorhexidine with chitosan provides a synergistic effect against E.faecalis.[7] Chitosan along with calcium hydroxide causes sustained release of calcium from calcium hydroxide and maintenance of alkaline environment for a period of 30 days.[8]

- ***Zinc oxide Nanoparticles***

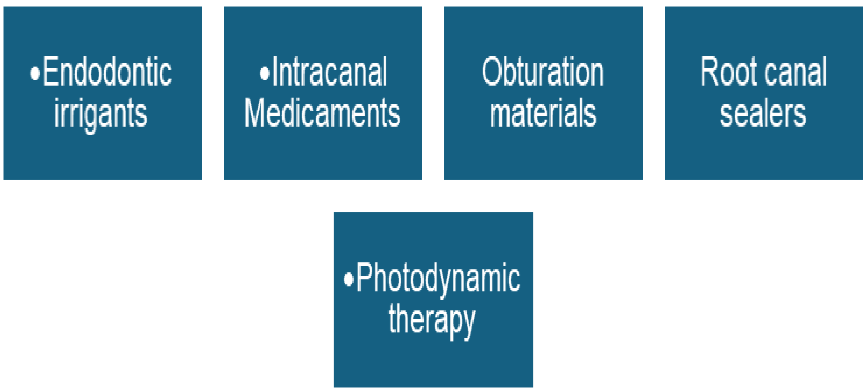
Zinc oxide nanoparticles affect the enzymatic activity of bacteria. Zinc oxide NPs can be used as irrigating solution, intracanal medicament and as sealers. The antibacterial activity of zinc oxide nanoparticles is like that of other types of nanoparticles. The bactericidal effect of zinc oxide nanoparticles is related to the size- the smaller the size, the higher will be the antibacterial effect and the production of reactive oxygen species such as hydrogen peroxide when in contact with an aqueous medium. ZnO-NPs used as an irrigant solution and when added to zinc oxide-based sealer have shown to decrease the number of colony forming units of the tested bacteria.[9]

- ***Copper Nanoparticles***

They exhibit potent anti- inflammatory, antimicrobial properties and are biocompatible. Positively charged Copper NPs attract negatively charged bacteria and accumulates on their cell membranes increasing the cellular permeability. This leads to the production of ROS, protein degradation and eventually bacterial cell death.

Clinically relevant applications in Endodontics

Nanoparticles can be used as



A. As irrigants or irrigant adjuncts

NP suspensions (AgNPs, ZnO NPs) mixed with NaOCl or used alone have been tested as irrigants. Combination with NaOCl often yields additive or synergistic reduction in CFU and biofilm biomass versus NPs alone, but NaOCl remains the gold standard for broad-spectrum tissue dissolution and biofilm reduction in many studies. Nanoparticles, especially AgNPs and chitosan nanoparticles are used as final irrigants to increase antibiofilm activity, penetrate dentinal tubules, and reduce bacterial viability compared with some conventional irrigants. Some formulations combine NPs with $Ca(OH)_2$ or with gels for sustained action. NP-containing finishing rinses or adjuncts have shown to improve reduction of mature biofilms and the evidence is promising in in-vitro and ex-vivo studies but clinical RCT data remain limited. A few studies report strong antibiofilm activity even at low AgNP concentrations (e.g., 0.01–0.02% AgNP formulations).[15]

B. Intracanal medicaments

Chitosan-based NPs, AgNP-loaded pastes, and nanocarrier-enhanced calcium hydroxide/antibiotic pastes have been studied as interappointment medicaments to reduce residual biofilm and prevent recolonization. Some formulations show prolonged antibiofilm activity in dentin blocks/ex vivo teeth. It is possible to combine calcium hydroxide with silver nanoparticles (size 20 nm), which have demonstrated enhanced antibacterial activity when used either alone or in conjunction with chlorhexidine.[16,17] As an intracanal medication, the commercially available NanocarePlus Silver and Gold (NanoCare Dental, Nanotechnology, Katowice, Poland) has demonstrated encouraging antibacterial qualities.

C. Sealers, obturation materials and coatings

Incorporation of NPs into sealers (e.g., chitosan, AgNPs) aims to prevent biofilm regrowth at the sealer–dentin interface and improve long-term outcomes. Several studies report reduced biofilm formation on NP-modified cements in vitro.[18,19]

D. Photodynamic & photoactivated NP adjuncts

Photosensitizer-loaded NPs and nano-carriers that enhance aPDT penetration have shown greater biofilm reductions than photosensitizer alone in ex vivo root canal models. Multifunctional NPs that combine penetration promoters with aPDT show promising results.[20]

Safety, Toxicity & Resistance Considerations

• ***Host Cytotoxicity Risks***

The release of metal ions (e.g., Ag^{+}, Zn^{2+}), reactive oxygen species (ROS) generation, and nanoscale particle dimensions can elevate cytotoxic effects on host cells such as fibroblasts and stem cells. Strategies like surface coatings and controlled-release systems offer mitigation potential, but their efficacy must be rigorously quantified.[21]

• ***Microbial Resistance & Environmental Impact***

Chronic, sublethal exposure to metallic nanoparticles may promote the emergence of metal-tolerant microbial strains and facilitate horizontal gene transfer. Additionally, the environmental persistence of these particles raises ecological concerns.

Responsible stewardship including optimized dosing and exposure control is essential to minimize unintended consequences

CONCLUSION

Persistence of biofilm is the major cause for the failure of endodontic treatment. Hence, effective management of biofilm is essential for the success of root canal therapy and prevention of recurrent infections.

Nanoparticles have emerged as a promising approach for biofilm removal due to their unique properties like small size, high surface area, and the ability to penetrate and disrupt biofilms effectively. Their antimicrobial properties make them highly effective against biofilm-forming microorganisms.

Despite these advancements, few challenges such as potential toxicity, cost-effective large-scale production and their environmental impact need to be addressed.

Overall, nanoparticles represent a versatile and innovative tool for combating biofilm-associated problems in endodontics.

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